Novel Gene Sequence Analysis

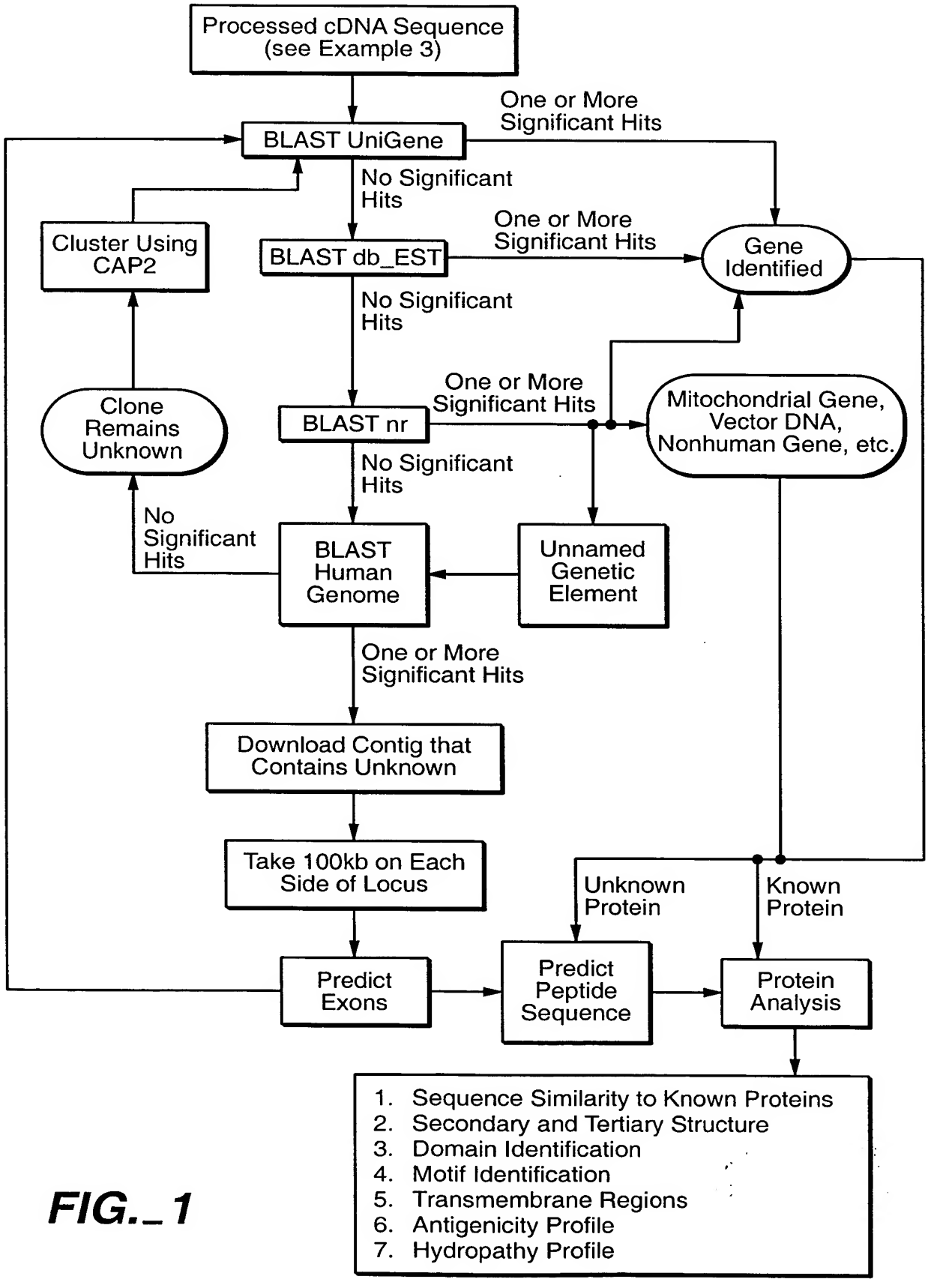

FIG._1

Automated Mononuclear Cell RNA Isolation Device

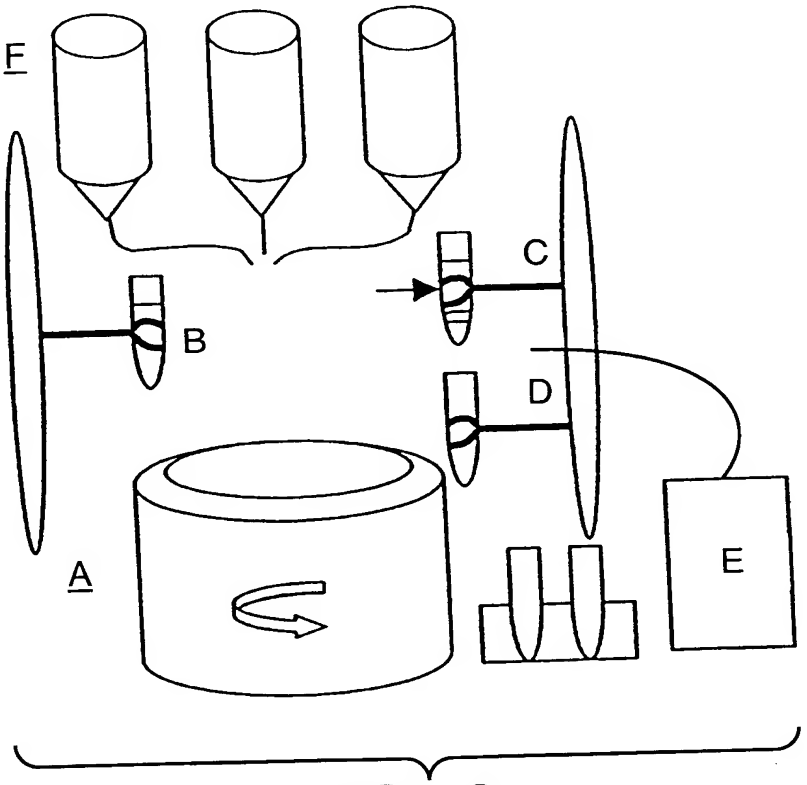

FIG._2

Kits for Discovery of, or Application of Diagnostic Gene Sets

A. Contents of kit for discovery of diagnostic gene sets

1. Sterile, endotoxin and RNAse free blood collection tubes (>10cc capacity)
2. Alcohol swabs, tourniquet, 18g needle and syringe (>10cc capacity)
3. Erythrocyte lysis buffer
4. Leukocyte lysis buffer
5. Substrates for labeling of RNA (may vary for various expression profiling techniques)
   For fluorescence cDNA microarray expression profiling:
   Reverse transcriptase and 10x RT buffer
   Poly-dT primer
   DTT
   Deoxynucleotides 100mM each
   RNAse inhibitor
   Cy3 and Cy5 labeled deoxynucleotides
6. cDNA microarrays containing candidate gene libraries
7. Cover slips for slides
8. hybridization chambers
9. Software package for identification of diagnostic gene set from data
   Contains statistical methods.
   Allows alteration in desired sensitivity and specificity of gene set.
   Software facilitates access to and data analysis by centrally located database server.
10. Password and account number to access central database server.
11. Kit User Manual

B. Contents of kit for application of diagnostic gene sets

1. Sterile, endotoxin and RNAse free blood collection tubes (>10cc capacity)
2. Alcohol swabs, tourniquet, 18g needle and syringe (>10cc capacity)
3. Erythrocyte lysis buffer
4. Leukocyte lysis buffer
5. Substrates for labeling of RNA (may vary for various expression profiling techniques)
   For fluorescence cDNA microarray expression profiling:
   Reverse transcriptase and 10x RT buffer
   Poly-dT primer
   DTT
   Deoxynucleotides 100mM each
   RNAse inhibitor
   Cy3 and Cy5 labeled deoxynucleotides
6. cDNA microarrays containing diagnostic gene sets
7. cover slips for slides
8. hybridization chambers
9. Software package for identification of diagnostic gene set from data
   Contains statistical methods.
   Allows alteration in desired sensitivity and specificity of gene set.
   Software facilitates access to and data analysis by centrally located database server
10. Password and account number to access central database server.
11. Kit User Manual

FIG._3

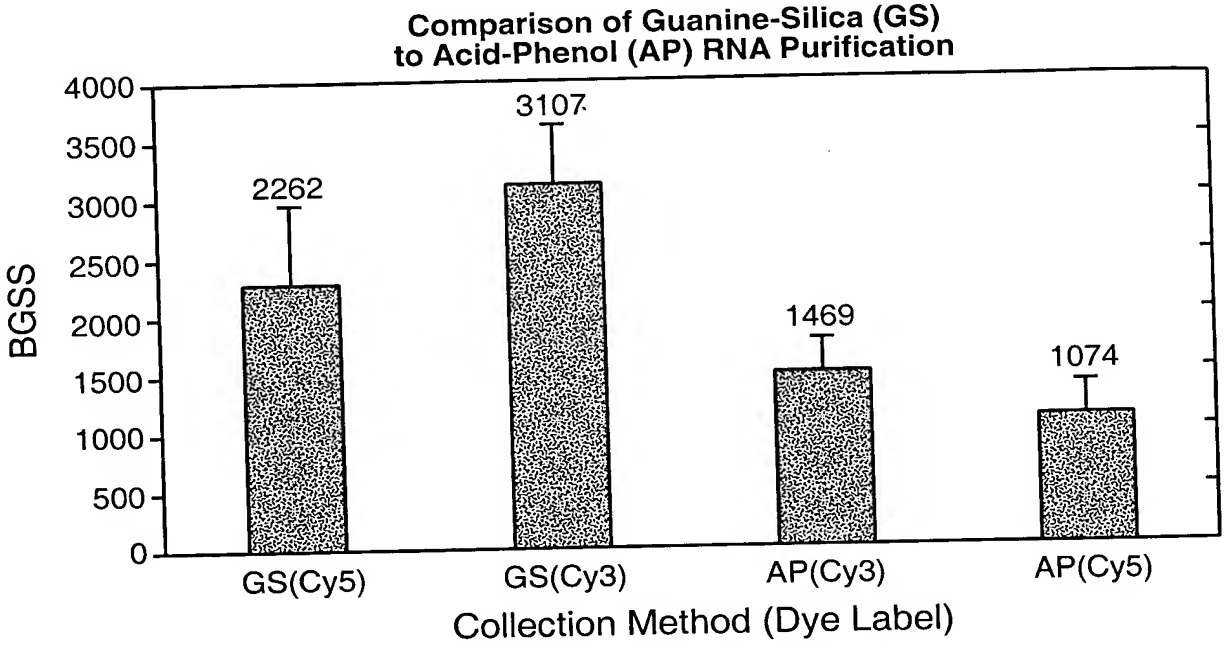
Comparison of Guanine-Silica (GS) to Acid-Phenol (AP) RNA Purification
BGSS
4000
3500
3000
2500
2000
1500
1000
500
0
2262
3107
1469
1074
GS(Cy5)
GS(Cy3)
AP(Cy3)
AP(Cy5)
Collection Method (Dye Label)

FIG._4

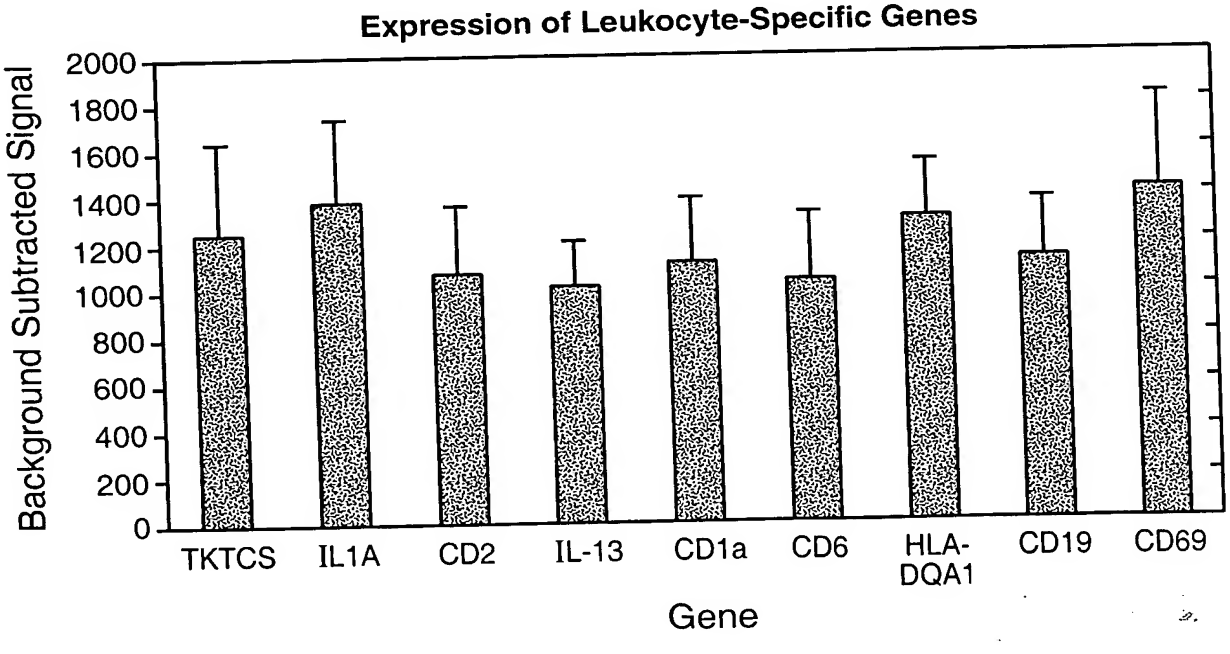
Expression of Leukocyte-Specific Genes
Background Subtracted Signal
2000
1800
1600
1400
1200
1000
800
600
400
200
0
TKTCS
IL1A
CD2
IL-13
CD1a
CD6
HLA-DQA1
CD19
CD69
Gene

FIG._5

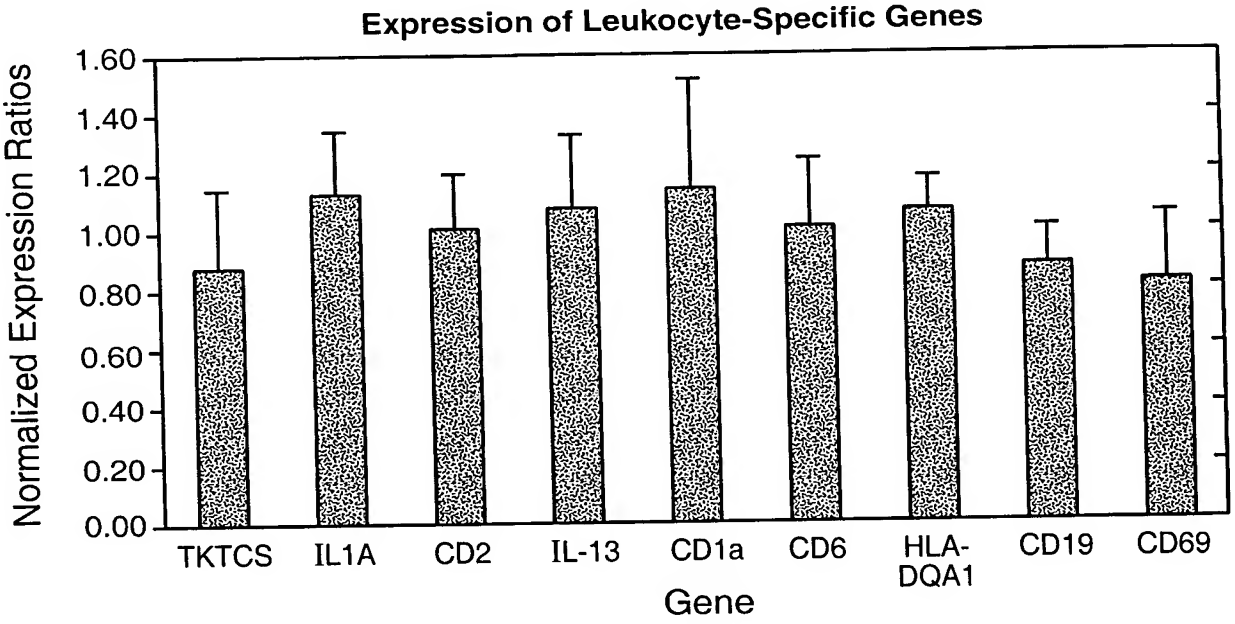
Expression of Leukocyte-Specific Genes
Normalized Expression Ratios
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
TKTCS
IL1A
CD2
IL-13
CD1a
CD6
HLA-DQA1
CD19
CD69
Gene

FIG._6

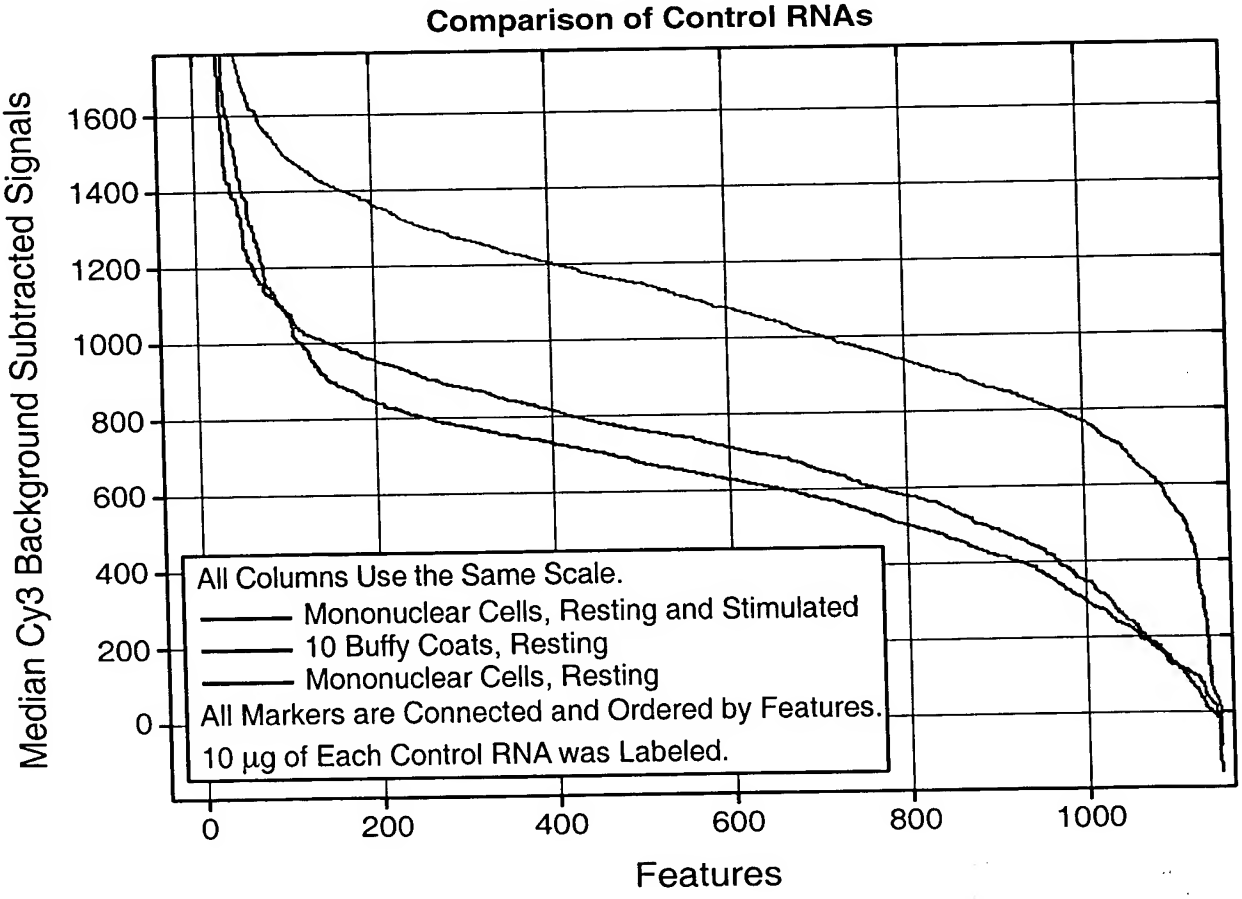
Comparison of Control RNAs
Median Cy3 Background Subtracted Signals
1600
1400
1200
1000
800
600
400
200
0
All Columns Use the Same Scale.
Mononuclear Cells, Resting and Stimulated
10 Buffy Coats, Resting
Mononuclear Cells, Resting
All Markers are Connected and Ordered by Features.
10 µg of Each Control RNA was Labeled.
0
200
400
600
800
1000
Features

FIG._7

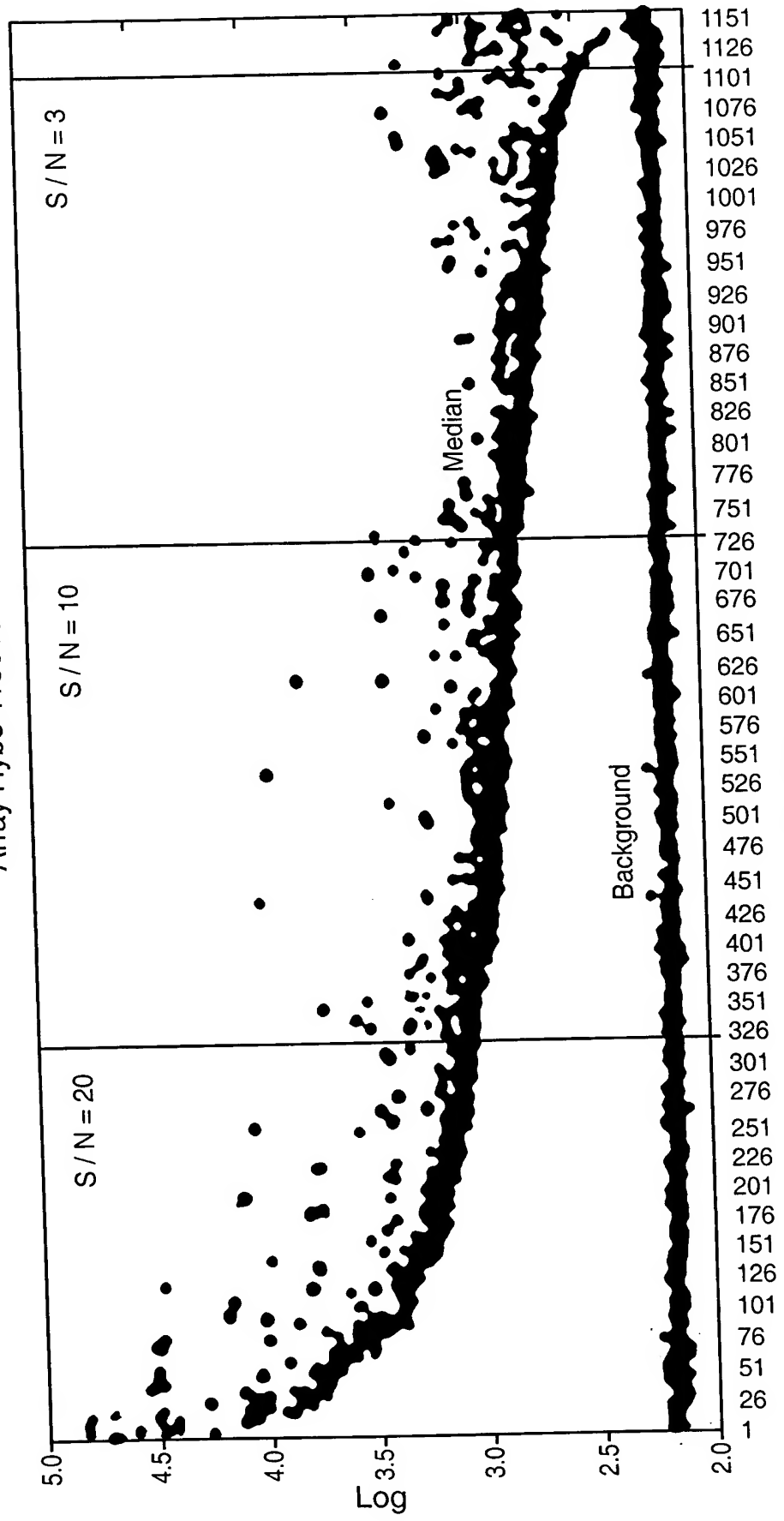
Log Expression of Each Probe Using the R50 Reference RNA. Probe Expression is Ordered by Signal to Noise, S / N, Decreasing from Left to Right.
Array Hybe 115018
S / N = 3
S / N = 10
S / N = 20
Median
Background
Probe #
1151
1126
1101
1076
1051
1026
1001
976
951
926
901
876
851
826
801
776
751
726
701
676
651
626
601
576
551
526
501
476
451
426
401
376
351
326
301
276
251
226
201
176
151
126
101
76
51
26
1
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Log

FIG._8

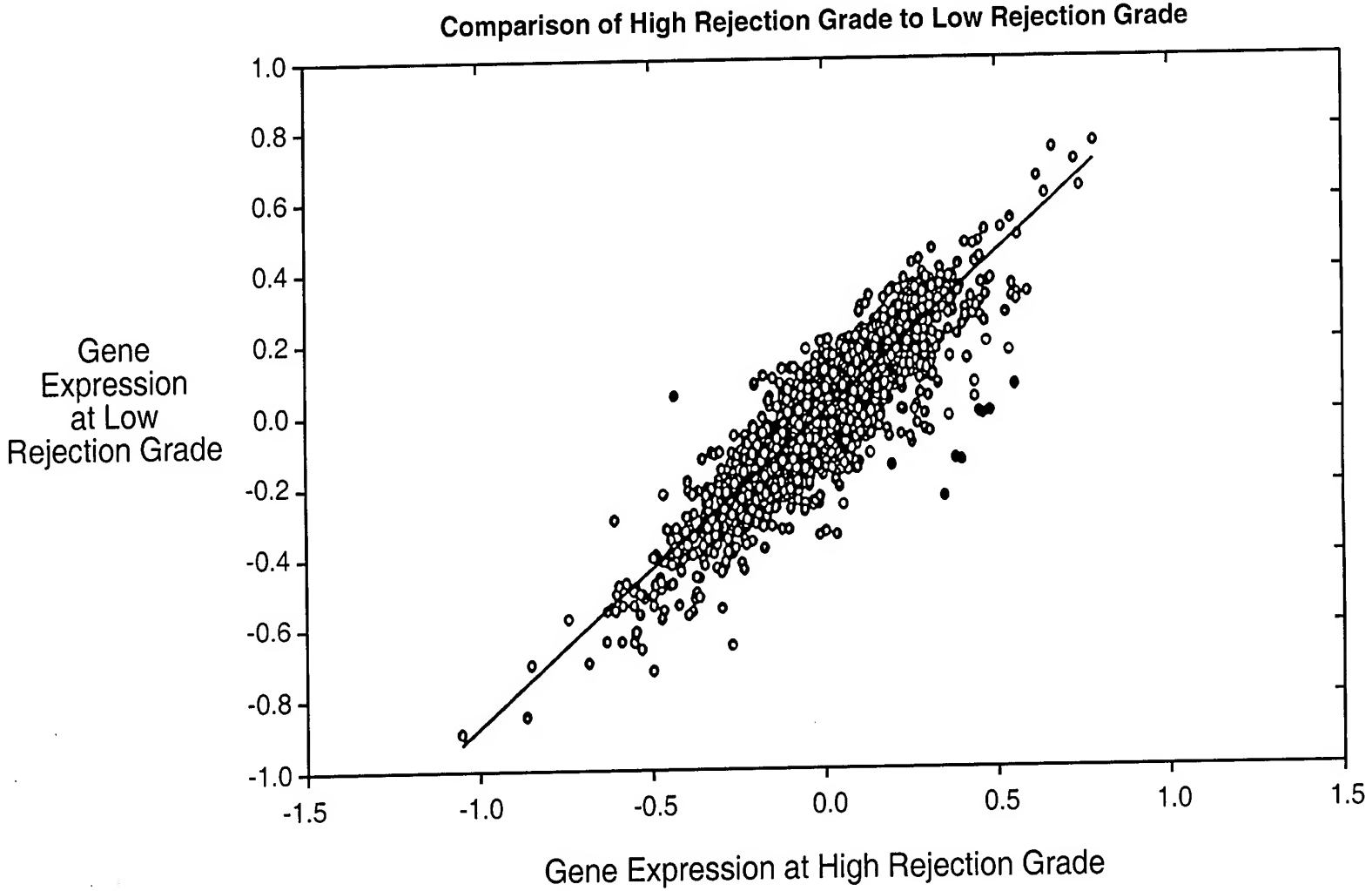

FIG._9

FIG._10A

Differential Gene Expression Between Grade 0 and 3A Samples:

Probe			Array 107742: Grade 0			
Acc#	Name	Oligo ID	F633 Median - B633	F532 Median - B532	Cy3 / Cy5 Ratio	SR: Scaled Ratio (g / r)
NM_003202	transcription factor 7 (T-cell specific, HMG-box) (TCF7),	2476	5558	1050	0.188917	0.710038
BE220959	major histocompatibility complex, class II, DQ beta 1 (HL	6025	1810	635	0.350829	1.318579
BE220959	major histocompatibility complex, class II, DQ beta 1 (HL	6025	1402	487	0.347361	1.305545
NM_002922	regulator of G-protein signalling 1 (RGS1), mRNA / cds=	2407	804	95	0.118159	0.444098
NM_001781	CD69 antigen (p60, early T-cell activation antigen) (CD6	2192	4121	405	0.098277	0.369371
NM_002341	lymphotoxin beta (TNF superfamily, member 3) (LTB), tr	2283	13488	3477	0.25556	0.960516
BE220959	major histocompatibility complex, class II, DQ beta 1 (HL	6025	1539	515	0.334633	1.257707
NM_001781	CD69 antigen (p60, early T-cell activation antigen) (CD6	2192	3850	386	0.10026	0.376823
U05040	far upstream element (FUSE) binding protein 1 (FUBP1	3581	4507	1119	0.24828	0.933154
X14008	nuclear receptor subfamily 4, group A, member 2 (NR4A	3729	1365	167	0.122344	0.459827
NM_003202	transcription factor 7 (T-cell specific, HMG-box) (TCF7),	2476	2716	486	0.17894	0.672539
AF035947	cytokine-inducible inhibitor of signaling type 1b mRNA,	642	9850	5254	0.533401	2.004771
NM_001781	CD69 antigen (p60, early T-cell activation antigen) (CD6	2192	3357	356	0.106047	0.398574
Y14737	mRNA for immunoglobulin lambda heavy chain / cds=(65	4905	1390	248	0.178417	0.670576
Y14737	mRNA for immunoglobulin lambda heavy chain / cds=(65	4905	1398	240	0.171674	0.645231
BC006402	mRNA for immunoglobulin lambda heavy chain / cds=(65	4481	1826	295	0.161555	0.6072
X57812	rearranged immunoglobulin lambda light chain mRNA / c	3761	6512	747	0.114711	0.431139
X57812	rearranged immunoglobulin lambda light chain mRNA / c	3761	6728	755	0.112218	0.421766
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3790	8572	1188	0.138591	0.520889
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3790	15538	2128	0.136955	0.514739
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3791	11974	1558	0.130115	0.489034
X57812	rearranged immunoglobulin lambda light chain mRNA / c	3761	6953	778	0.111894	0.420551

FIG._10B

Array 107739: Grade 3A				Ratio of SRs	
F633 Median - B633	F532 Median - B532	Cy3 / Cy5 Ratio	SR: Scaled Ratio (g / r)	Grade 0 / 3A	Grade 3A / 0
5827	358	0.061438	0.219793	3.23048873	0.30955069
2150	252	0.117209	0.419312	3.14462275	0.31800317
2121	247	0.116455	0.416612	3.13371968	0.31910959
1884	75	0.039809	0.142415	3.11833431	0.32068403
7385	254	0.034394	0.123043	3.00195843	0.33311587
29882	2727	0.091259	0.326476	2.94207495	0.33989617
1942	237	0.122039	0.436591	2.88074602	0.3471323
7705	282	0.0366	0.130934	2.87796556	0.34746767
2390	220	0.09205	0.329306	2.83369583	0.35289603
9541	434	0.045488	0.162731	2.82568319	0.35389672
5310	356	0.067043	0.239845	2.80405488	0.3566264
969	197	0.203302	0.727307	2.75642938	0.36278818
5963	246	0.041254	0.147586	2.70062225	0.37028503
6561	5767	0.878982	3.144527	0.21325167	4.68929496
7159	6112	0.853751	3.054262	0.21125576	4.73359863
2973	2498	0.840229	3.005889	0.20200364	4.95040579
27381	17730	0.647529	2.316513	0.18611538	5.37301111
28820	18636	0.646634	2.313311	0.18232143	5.48481867
17322	13892	0.801986	2.869076	0.18155283	5.50803866
17637	14245	0.807677	2.889436	0.17814525	5.61339689
24261	18761	0.773299	2.766449	0.17677319	5.65696646
27621	18560	0.671952	2.403886	0.1749461	5.71604612

X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3791	10805	1411	0.130588	0.49081
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3790	11246	1453	0.129201	0.4856
AF067420	SNC73 protein (SNC73) mRNA, complete cds / cds=(39	4399	2654	243	0.09156	0.344125
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3791	10909	1370	0.125584	0.472005
AF067420	SNC73 protein (SNC73) mRNA, complete cds / cds=(39	4399	1959	181	0.092394	0.34726
AF067420	SNC73 protein (SNC73) mRNA, complete cds / cds=(39	4399	2558	215	0.08405	0.315899
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4474	7538	684	0.09074	0.341044
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4474	8662	780	0.090048	0.338444
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4474	7183	608	0.084644	0.318133
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4475	8986	851	0.094703	0.355938
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4476	11118	1023	0.092013	0.345828
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4475	7428	730	0.098277	0.36937
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4476	10413	933	0.0896	0.336757
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4475	5841	484	0.082863	0.311436
AF067420	SNC73 protein (SNC73) mRNA, complete cds / cds=(39	4398	7960	645	0.08103	0.304549
AF067420	SNC73 protein (SNC73) mRNA, complete cds / cds=(39	4398	11959	992	0.08295	0.311765
AF067420	SNC73 protein (SNC73) mRNA, complete cds / cds=(39	4398	6161	447	0.072553	0.272689

FIG._10C

17533	14334	0.817544	2.924735	0.16781337	5.95900079
17074	13863	0.811936	2.904673	0.16717875	5.9816215
37518	21610	0.57599	2.060585	0.16700357	5.98789603
21668	18561	0.856609	3.064488	0.15402406	6.4924922
30274	19369	0.63979	2.288826	0.15171979	6.59109804
36161	21936	0.60662	2.170163	0.14556481	6.86979225
6038	4037	0.668599	2.391889	0.14258368	7.01342553
4339	2975	0.685642	2.45286	0.13797951	7.24745312
5521	3909	0.708024	2.532931	0.12559874	7.96186351
1587	1275	0.803403	2.874145	0.12384126	8.0748531
871	682	0.783008	2.801184	0.12345771	8.09993947
1049	890	0.848427	3.035218	0.12169477	8.21727973
625	486	0.7776	2.781837	0.12105563	8.2606647
1694	1344	0.793388	2.838319	0.10972555	9.11364747
22985	18694	0.813313	2.909599	0.10467052	9.55378803
14170	12597	0.888991	3.180333	0.0980291	10.2010527
16180	14148	0.874413	3.128181	0.08717165	11.4716196

FIG._10D

FIG._10

FIG._10A	FIG._10B
FIG._10C	FIG._10D